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Flame Photometric Determination of Magnesium in Biological Samples

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To the Graduate Council:

I am submitting herewith a thesis written by Carole Claudia Ewing entitled "Flame Photometric Determination of Magnesium in Biological Samples." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

Frances A. Schofield, Major Professor

We have read this thesis and recommend its acceptance:

Ada Marie Campbell, John T. Smith

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

THE UNIVERSITY OF TENNESSEE

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ABSTRACT OF EDUCATIONAL RESEARCH STUDY COMPLETED

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Abstract approved by Frances A. Schofield
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The study was undertaken to investigate flame photometric determinations of magnesium in solutions of ash from biological samples collected during metabolic balance experiments. Two general methods for elimination of interference by other ions were studied. In one adverse effects were eliminated by addition of anions and cations to the standard solutions in amounts comparable to the levels in samples to be analyzed. However, this method was discontinued because the atomizer burner of the flame photometer became too badly clogged for further use. Poorly volatilized calcium sulfate and phosphate may have been responsible.

The other method depends on removal of interfering anions by precipitation and addition of interfering cations to the standard solution. The first procedure investigated involved removal of phosphate and sulfate ions by precipitation with stannic chloride and addition of calcium, of calcium and sodium, and finally of calcium, sodium, and potassium to the standards to compensate for the varying amounts present in the samples. Reproducibility of analysis, recovery of added magnesium, and agreement of values obtained by the flame photometric and a colorimetric determination were criteria for evaluation of the method. Standard curves were reproducible in all series of determinations but replicate analyses of solutions were in poor agreement. Apparent magnesium concentration of solutions of ash and recovery of added standard were dependent on the amount of magnesium in the diluted sample analyzed. Agreement of magnesium concentrations obtained by the two methods was satisfactory only for the series in which calcium alone was added to the standard solutions.

Precipitation of phosphate and sulfate ions with barium and ferric chlorides from standards and samples was the other procedure studied. In one series of determinations calcium, sodium, and potassium chlorides were added to the standards to compensate for the amounts found in the samples. Reproducibility and recovery of added magnesium were no better than those obtained by previous determinations. Influence of the concentration of the

solution analyzed upon the apparent magnesium concentration of the original solution of ash was not eliminated. Results obtained by the flame photometric method were in every case much less than those from colorimetric determinations.

The procedures for flame photometric determination of magnesium in ash from food, feces, and urine samples were generally unsatisfactory judged by all of the criteria studied. Processes required for removal of the interfering anions and addition to the standards of interfering cations were so time consuming that the flame photometric method studied had little advantage over colorimetric methods in this respect.

July 15, 1963

To the Graduate Council:

I am submitting herewith a thesis written by Carole Claudia Ewing entitled "Flame Photometric Determination of Magnesium in Biological Samples." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

Frances A. Schofield
Major Professor

We have read this thesis and recommend its acceptance:

Edna Marie Campbell

John T. Smith

Accepted for the Council:

Dean of the Graduate School

FLAME PHOTOMETRIC DETERMINATION OF MAGNESIUM IN BIOLOGICAL SAMPLES

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Carole Claudia Ewing

August 1963

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CHAPTER I

INTRODUCTION

Mineral metabolism in human subjects is one aspect of the research in progress in the Nutrition Department at the University of Tennessee. When a study of magnesium metabolism was initiated a colorimetric method was sought for magnesium determinations in samples collected in the metabolic balance studies. A thiazole yellow colorimetric procedure, developed for analysis of plant tissue, was modified for analysis of ash solutions of food, feces, and urine. The method has proved thoroughly satisfactory and is fairly rapid. However, because of the large number of samples to be analyzed, use of a considerably faster flame photometric analysis seemed desirable if conditions suitable for magnesium determination in the biological materials could be established.

Other ions present in the ash from food, feces, and urine interfere with magnesium analysis by the flame photometric method. The purpose of the work reported in this paper was to investigate procedures available for elimination of interference by sulfate, phosphate, calcium, sodium, and potassium in an attempt to find conditions which would permit satisfactory magnesium determinations

in the types of materials to be analyzed. Reproducibility of analyses made at two different sample concentrations and in different series of determinations, recovery of magnesium added to solutions of ash, and agreement of results with those obtained by colorimetric analysis of the same samples were criteria for evaluation of procedures.

CHAPTER II

REVIEW OF LITERATURE

Up to the middle of the twentieth century, analysis of biological materials for magnesium was made by the tedious gravimetric method. The determination is extremely time consuming and subject to several sources of error. Development of several colorimetric procedures provided much more rapid methods of analysis (Thomas, 1958; Andrews, 1960). However, the various methods described were frequently satisfactory only in the hands of the authors. As a result conflicting evaluations of the colorimetric procedures are found in the literature. Use of the rapid flame photometric method for magnesium determination has received attention also (Hathaway, 1962).

Flame spectrophotometry has been in use nearly 100 years for the qualitative identification of metals but prior to 1929 the literature disclosed little work on the use of flame spectrophotometry for quantitative analysis (Gilbert et al., 1950). Since that time spectroscopic determination which eliminates or reduces chemical treatment of samples has attracted the attention of analysts (West et al., 1950). With the development of the photomultiplier tube which might

permit determination of those elements that emit radiation weakly in the flame (Brealey and Ross, 1951), interest of numerous investigators has been directed toward adaptation of the flame photometric method to magnesium determination (Knutson, 1957; Manna et al., 1957; Knox, 1960; Alcock et al., 1960; Andersen et al., 1962).

Flame photometers such as those manufactured by Perkin-Elmer, Baird Associates, and Beckman Instruments are commercially available (Margoshes and Vallee, 1955). The instrument consists essentially of 3 parts: (1) the pressure regulators or flow meters for the fuel gases, (2) the atomizer, (3) the burner, (4) the optical system, (5) the photosensitive detector, and (6) the instrument for indicating or recording the output of the detector (Willard et al., 1958).

The flame photometric method can be used to analyze small quantities of material or determine low concentrations of most of the elements which exhibit flame spectra. Two advantages of the method are precision and speed (Caton and Bremner, 1954). However, this method generally is not credited with accuracies exceeding $\pm$ 3.5 per cent of the amount present (Dean and Thompson, 1955). In practice, optimal precision and sensitivity are achieved only when the many factors affecting emission of light and its measurement are recognized and controlled. Some factors influencing precision of measurements are: (1) the burner, (2) components of the flame, (3) monochromator,

(4) detector, (5) amplifier, (6) composition of the samples, and (7) the solvent used (Margoshes and Vallee, 1956).

The principle cause of difficulty with flame photometric analysis is direct interference effects of other elements such as nonbackground radiation which can occur when an emission line falls within an emission band of another ion or when an intense line emission is adjacent (Gilbert et al., 1950). In addition background radiation seriously affects determination of weak emitters. In the case of magnesium, the flame background at 285.2 millimicrons (hereafter referred to as mμ) is extremely complex. An intense sodium line occurs at 285.3 mμ (Alcock et al., 1960) and a calcium oxide band at 554 mμ (Willard et al., 1958). Background radiation increases also with the proportion of acetylene in the fuel used for the flame, but this can be kept at a minimum by reduction of the slit width (Knutson, 1957).

Several investigators have reported interference by a number of anions and cations. Baker and Johnson (1954) obtained results which indicated that pyrosulfate and pyrophosphate ions may be responsible for flame anomalies which decrease the calcium flame intensity. Dippel et al. (1954) found by plotting emission intensity of calcium and of magnesium at constant concentrations against the concentration of phosphate that the emission intensity passed through

a minimum after which it increased until enhancement occurred at phosphate concentrations exceeding one molar. Willard et al. (1958) stated that phosphate and sulfate could form compounds with calcium which had comparatively high melting and boiling points so that calcium ions could not be made available for excitation. Margoshes and Vallee (1955) reviewed evidence which showed calcium sulfate and calcium phosphate to be less volatile than calcium chloride, thereby suggesting possible interference. Elimination of interference may be accomplished by dilution of the sample until the effect is negligible, by addition of salts to the standard to approximate the composition of the sample, or by removal of the interfering substances (Caton and Bremner, 1954).

Permissible concentration ratios between interfering cations and elements to be determined generally increase when solution concentrations decrease. Conrad and Johnson (1950) found that interferences of cationic nature were greatly reduced at high dilutions. Calcium interferes in magnesium determination by enhancement of magnesium emission and sodium and potassium by increasing background radiation (Knox, 1960). Dippel et al. (1954) also found cationic interference with calcium and magnesium analysis minimized at high dilutions. The hydrogen ion effect was established as insignificant by Baker and Johnson (1954) but the

OH band spectrum was found to interfere with the magnesium line (Willard et al., 1958).

Addition to the standard of ions to approximate concentrations in solutions of biological materials was suggested by Alcock et al. (1960). In this way, enhancement or suppression of magnesium emission by the interfering ions in the standards corresponds to similar effects in biological materials and errors introduced by varying levels of interfering ions in the samples are reduced. The authors found that addition of a compensating solution to the magnesium standards permitted satisfactory calcium and magnesium determinations in plasma, urine, and ash solutions from feces, using a Zeiss flame spectrophotometer PMQII. West et al. (1950) also believed that if high constant amounts of diverse cations were added to the standard solutions small concentration variations in the samples would be without effect upon the emission strength of the metallic ion in aqueous solutions.

Elimination of anion interference has been discussed by several investigators. Margoshes and Vallee (1955) suggested removal by precipitation and Dippel et al. (1954) recommended removal by an anion exchange resin prior to analysis. Leyton (1954) stated that complete elimination of phosphate was necessary if reliable calcium determinations were to be made with the flame photometer.

Although this technique had not been used in his laboratory, he felt that an indirect separation by calcium adsorption and subsequent elution from a cation exchange resin column was a promising procedure. Margoshes and Vallee (1955) advocated removal of phosphate and sulfate by precipitation with stannic chloride. Stannic sulfate and phosphate precipitate within a few minutes, followed by the hydrolysis of excess stannic chloride to form gelatinous stannic acid which is readily removed by centrifugation. In this way all excess reagent is removed from the solution. Determinations of calcium in biological fluids by this procedure appeared satisfactory and 95 per cent recovery of added calcium was obtained. Shaw and Veal (1956) eliminated phosphate interference in magnesium and calcium determinations on soil extracts by precipitation with ferric chloride. A modification of this method by Steyges (1962) has been used successfully for analysis of plant tissues from which phosphate and sulfate were removed by precipitation with ferric and barium chlorides prior to analysis.

The use of organic solvents has contributed to the utility of flame spectrophotometric methods of analysis (Carnes, 1961). It has been reported that with the use of organic solvents, the possibility of bringing more trace elements within the range of measurement of the flame photometer and the use of smaller samples now exists (Kingsley

and Schaffert, 1952; 1954). These authors were able to make flame photometric determinations of sodium, potassium, and calcium on extremely small biological specimens. Different solvents were found to produce varying levels of emission enhancement, acetone being the most effective. Small amounts of alkali and alkaline earth elements in lubricating oils were successfully determined through the enhanced emission in solutions of organic solvents (Conrad and Johnson, 1950). The organic solvent acts as a fuel and by its combustion increases the temperature of the flame whereas energy must be spent in heating and evaporating water from aqueous solutions aspirated into the flame (Dean and Lady, 1955; Knox, 1960).

One other method for at least partial elimination of interfering anions has been studied by several authors. This depends upon separation of a cation from anions and some of the interfering cations by extraction. The particular ion is made soluble in various organic solvents by the use of an appropriate chelating agent and removed from the aqueous solution (Dean and Lady, 1955; Dean and Cain, 1957). Carnes (1961) showed such a procedure was satisfactory for yttrium determination and Knox (1960) suggested application of the technique in magnesium analysis.

Use of atomic absorption spectra presents a new and promising method of photometric analysis with advantages over the emission

methods. Theoretically, the method should be less susceptible to interelement effects (Walsh, 1955). Sensitivity is so great that interference can be largely eliminated by extreme dilution (Parker, 1963). Because analysis of biological materials for magnesium by flame spectrophotometry is generally unsatisfactory, use of the atomic absorption method for magnesium has received considerable study. Allan (1958) found reproducibility of magnesium analysis in plant material and soil extracts to be within one per cent. He stated that provided sufficient regard was paid to physical properties which could influence the atomization and to chemical constituents which could combine with magnesium in the flame, the method should be applicable to any solution containing magnesium. Dawson and Heaton (1961) found reproducibility of analyses of biological materials by the atomic absorption method to be within one per cent on 40 different samples. Work completed by Willis (1960) confirmed the report by Allan that the presence of sodium, potassium, calcium, and phosphate did not affect the magnesium determination. Atomic absorption determinations of magnesium may prove especially beneficial in analysis of biological materials.

In the opinion of Hathaway (1962), no satisfactory method for the determination of magnesium in biological materials has been reported. The colorimetric procedure in use in the Nutrition laboratory

at the University of Tennessee has given excellent reproducibility and satisfactory recovery of added magnesium in the analysis of solutions of ash from food, feces, and urine (Andrews, 1960). Nevertheless, the possibility that flame photometric analysis might be more rapid, if a procedure suitable for magnesium determination in these samples could be found, prompted the studies reported in this paper.

CHAPTER III

PROCEDURE

A study of flame photometric determination of magnesium in biological materials was made using a Beckman Model DU spectrophotometer with photomultiplier and flame attachments. The sample aspirator and excitation source was a model 4030 acetylene atomizer burner. Although the acetylene-oxygen flame has the disadvantage of considerably greater background interference than the hydrogen-oxygen flame, use of the hotter flame is necessary for excitation of weakly emitting ions such as magnesium.

The instrument was adjusted for maximum transmittance dial reading at 598 mμ with tap water from which air had been removed by boiling. This setting on the wavelength dial corresponded to the 589 mμ sodium line. Final mirror adjustment reduced flame background to its minimum. The peak of the magnesium line was located by noting maximum deflection on the null meter when a solution containing 1000 parts per million of magnesium was sprayed while rotating the wavelength dial from 284-287 mμ at a slit width of 0.05-0.1 millimeters (hereafter referred to as mm). The dial reading of the instrument corresponding to the 285.2 mμ magnesium line was 285.9. This

setting was verified repeatedly and could be located with a slit width less than 0.02 mm.

Instrument dial settings found most satisfactory and used throughout the study unless otherwise indicated were:

Selector switch	.1
Sensitivity	5.0
Zero suppression	3.0
Wavelength	285.9 mμ
Slit width	.025 mm
Oxygen	15.0 pounds per square inch (hereafter referred to as psi)
Acetylene	3.0 psi.

The power supply unit was not turned off between determinations and the selector switch was left on "check." Nevertheless a warm-up interval of at least one hour prior to a series of determinations proved necessary for smooth operation.

For each series of analyses, a standard curve was prepared using a blank and standard solutions of magnesium sulfate supplying the ion at concentrations of 5, 10, 15, 20, 30, and 40 micrograms (hereafter referred to as μg) per milliliter (hereafter referred to as ml). At intervals during a series of determinations the transmittance

dial was set at 100, the most concentrated standard solution aspirated and the null meter returned to zero by slight adjustment of the sensitivity control knob.

The materials to be analyzed were composites of food, feces, and urine which were collected during a metabolic balance study at the University of Tennessee. Aliquots of the composites were weighed into silica dishes, dried under infrared lamps, heated over a flame until volatile matter was removed, and ashed in a muffle furnace at 550 degrees centigrade (hereafter referred to as ^{o}C) for 15-24 hours. Residues were cooled, moistened with demineralized water and treated with a small amount of concentrated hydrochloric acid to dissolve the ash. After further dilution, the sample was filtered through ashless filter paper into a volumetric flask and diluted to volume. The calcium content of the particular composite determined the amount of slurry ashed. Aliquots of these solutions were further diluted with demineralized water to contain less than 40 µg magnesium per ml. Samples chosen for use in this study included solutions of ash from 4 food, 4 feces and 4 urine composites. All had been analyzed for magnesium by the Tennessee modification (Thomas, 1956; Andrews, 1960) of the Young and Gill (1951) colorimetric method and for calcium by the oxalate-permanganate procedure (AOAC, 1960, p. 160). Phosphorus levels had been

determined by the microchemical method (AOAC, 1960, p. 644) modified for determination with a photoelectric colorimeter. The magnesium concentration of the ash solutions determined by colorimetric analysis served as a reference to aid in evaluation of results obtained in the present study. Reproducibility of analyses at different dilutions and at different times, recovery of added standard at varying dilutions, and agreement with colorimetric determinations were criteria of adequacy of the flame photometric method investigated.

Four procedures suggested for preparation of samples containing interfering anions and cations are elimination of interference by dilution of the sample until interference is negligible, addition of salts to the standards to approximate the composition of the sample, removal of interfering substances, and extraction of the cation with an organic solvent. The dilution method was not feasible because the calcium and phosphorus content of solutions from food and feces was several times the magnesium content of the samples. Extraction seemed to present error problems that made it appear undesirable also. Therefore, the other possibilities were chosen for study.

The procedure selected for initial study was that of Alcock et al. (1960). In the colorimetric determination of magnesium, interference by many of the inorganic ions present in biological materials is eliminated by addition to standards and samples of a

compensating solution supplying such ions at the maximum effective concentration. Because of experience with the effectiveness of a compensating solution in the colorimetric procedure, the flame photometric method described by Alcock et al. (1960) for determination of magnesium in biological materials seemed likely to be suitable, also.

A standard solution of magnesium was prepared from $MgSO_4 . 7H_2O$ which had been dried at 300°C in a muffle furnace for 7 hours; placed in a 100°C oven for at least one hour; cooled and weighed; returned to the furnace for one hour; cooled as before and reweighed. A 1.2375 gram (hereafter referred to as g) sample of the anhydrous salt was dissolved in distilled water and diluted to 250 ml to give a standard solution containing one milligram (hereafter referred to as mg) of magnesium per ml of solution. The compensating solution described by Alcock et al. (1960) supplied in the magnesium standard solutions calcium, sodium, potassium, chloride, sulfate, and phosphate in concentrations corresponding to maximum amounts found in normal human plasma, feces, or urine.

For the present study, standard solutions of magnesium were prepared containing 0, 5, 10, 15, 20, 30, 40 µg magnesium per ml with the same amount of compensating solution in each. Analysis of these gave the standard curve for each determination. Analyses were made on two consecutive days. By the end of the second series

of determinations the rate of aspiration of the samples was noticeably reduced and small lumps of black and white materials popped out of the burner at intervals. The evidence that non-volatilized material was clogging the burner indicated that the method was unsuitable with the type of burner available and the study of this procedure of necessity was discontinued.

The other general method used for elimination of interference is removal of the offending ions by precipitation. The fact that sulfate and phosphate reduce emission of calcium in the flame by formation of compounds with extremely high melting and boiling points suggested that the solid material deposited in the burner might well be calcium sulfate and phosphate mixed with carbon. Removal of these anions appeared the next step in the attempt to develop a procedure for magnesium determination.

Margoshes and Vallee (1955) proposed precipitation of phosphate and sulfate with stannic chloride. Since excess stannic ion is removed by hydrolysis and precipitation of stannic acid, the method has the advantage of avoiding cation contamination. This simple method to precipitate phosphate and sulfate ions from the solutions of ash was instituted. The amount of stannic ion necessary to react with the phosphate and sulfate in the samples was calculated, assuming the sulfate content to be similar to that reported by Macy (1942)

and using the phosphate concentration already determined. A solution of stannic chloride in 95 per cent ethyl alcohol was prepared containing 1.3380 g $SnCl_4 \cdot 5H_2O$ per 100 ml. This solution furnished 0.46 mg stannic ion per ml. Aliquots supplying stannic ion needed for precipitation of maximum amounts of sulfate and phosphate probably present in any diluted ash solution were added to equal volumes of all. Solutions were allowed to stand at least 4 hours for precipitation, centrifuged at 2000 revolutions per minute (hereafter referred to as RPM) for 15 minutes in an International Centrifuge Model V, size 2, and supernatants saved for analysis. Standard solutions were prepared each containing .02 mg calcium and 0-40 µg magnesium per ml. Although no phosphate was present in these solutions and the sulfate level was low, an amount of the ethanol-stannic chloride solution equal to that used per ml of ash solution was added to insure constant dilution of samples and standards. Precipitation and centrifugation were carried out in exactly the same manner as in the samples. Supernatants were aspirated in the flame to obtain points for a curve and sample readings.

In a preliminary trial, completeness of precipitation was verified by addition of a second aliquot of stannic chloride solution. The gelatinous precipitate which again formed evidently consisted entirely of stannic acid since except for slightly reduced transmittance

dial reading resulting from dilution, neither the standard curve nor the analyses were altered by the second precipitation. In all subsequent analyses one treatment with stannic chloride was considered adequate for complete removal of phosphate and sulfate ions.

By this method, phosphate- and sulfate-free diluted solutions of ash from food, feces, and urine were prepared containing magnesium ion in concentrations of 5-40 μg per ml. For each sample of all types of solution, the magnesium concentration of one was double that of the other. Recovery samples were prepared similarly containing 5 or 10 μg of added magnesium per ml of the more dilute solutions. Standard readings and sample analyses were carried out on 4 separate occasions. Lack of reproducibility of analyses and unsatisfactory recovery of added standard indicated that other ions present in varying amounts in the solutions of ash were interfering with magnesium determination. Since background radiation is increased by sodium ions, the relatively large amounts of sodium presumably present in solutions from food and urine were suspected of being responsible for the lack of reproducibility.

In the next series of experiments, influence of sodium ion concentration on sample analyses was studied. Standard solutions were prepared containing the same amounts of calcium and magnesium as before but with sodium chloride added. Sodium ion

concentrations were .03 or 0.3 mg per ml of final solution. Phosphate and sulfate ions were removed by precipitation as before from standards and samples. In an attempt to reduce further the background radiation, the slit width was reduced to .0125 mm. At the same time oxygen pressure was lowered to 13 psi to produce a somewhat hotter flame. Samples at two dilutions, and recovery samples were read as before against the new standard solutions. Determinations were made on three separate occasions. Finally, samples were read against standard solutions containing potassium chloride, also. Each solution, prepared as before, contained .02 mg calcium, 0.3 mg sodium, and .04 mg potassium with 0-40 µg magnesium per ml. Steps in the analysis were the same as those followed in all previous series.

Reproducibility of analysis and recovery of added magnesium were studied in another series of determinations in which phosphate and sulfate were removed using ferric and barium ions as precipitating agents. Solutions of barium chloride and ferric chloride supplying the ions at levels needed for precipitation of maximum amounts of sulfate and phosphate estimated in the diluted ash solutions were added in equal volumes per ml to all samples. Solutions were made slightly alkaline by the addition of ammonium hydroxide. Standard solutions were prepared containing .02 mg calcium, 0.3 mg sodium,

and .04 mg potassium, and 0-40 µg magnesium per ml. Since the only anions present were sulfate and chloride, addition of ferric ion was omitted. The same volumes of barium chloride and ammonium hydroxide used in each solution of ash were added to the standard solutions together with a volume of demineralized water equal to the amount of ferric chloride solution placed in the samples. Following an interval of about 12 hours, the precipitate was removed by centrifugation. The phosphate- and sulfate-free solutions of ash and ash solutions containing added magnesium were analyzed against the standards in 4 separate determinations.

Reproducibility of analyses under each set of conditions was studied. Apparent magnesium concentrations of the original solutions of ash were compared with those obtained by colorimetric analyses.

CHAPTER IV

RESULTS AND DISCUSSION

Calcium interferes with flame photometric determination of magnesium by enhancement of emission and the effect varies with the concentration of both magnesium and calcium in the solution. Because marked differences in calcium concentrations in solutions of food, feces, and urine existed, it seemed advisable to begin the study by using standard solutions which contained an amount of calcium comparable to the maximum amount present in the diluted solutions of ash. Calcium chloride was added to all standard solutions to furnish .02 mg calcium per ml.

Preliminary study of emission radiation obtained when the standard solutions of magnesium were aspirated into the oxy-acetylene flame showed that the relationship between emission and concentration was not linear and that the increase in emission with increasing concentration rapidly decreased above 20 µg magnesium per ml. Consequently in this study, all samples were diluted to furnish low concentrations of magnesium ions and the maximum concentration of the standards was 40 µg per ml. With one exception, in all the studies to be discussed interfering phosphate and sulfate

ions were removed by the addition of stannic chloride solution to standards and ash solutions prior to analysis.

A typical standard curve for magnesium solutions containing .02 mg calcium per ml is shown in Figure 1. Each value used for the curve represents the mean of 4 separate determinations. The replicate readings for a particular solution were in good agreement.

When sodium chloride as well as calcium chloride was added to standard solutions, sensitivity appeared to be slightly increased at magnesium concentrations below 20 µg per ml but was unaffected at higher levels. Reproducibility continued satisfactory. At the time potassium chloride as well as calcium and sodium chlorides was added to all standard solutions the oxygen pressure in the fuel was reduced in an attempt to increase magnesium radiation emission by using a slightly hotter flame. The slit width in the instrument was decreased at the same time in the hope that a decrease in background radiation effect might be obtained. Simultaneous introduction of changes in physical conditions and in composition of standards was made because preliminary trials showed that decrease in oxygen pressure and slit width produced no improvement in reproducibility of sample analysis. Under these conditions, little change in the shape of the standard curve was found in the concentration range studied. The steepest part of the curve was still found at magnesium

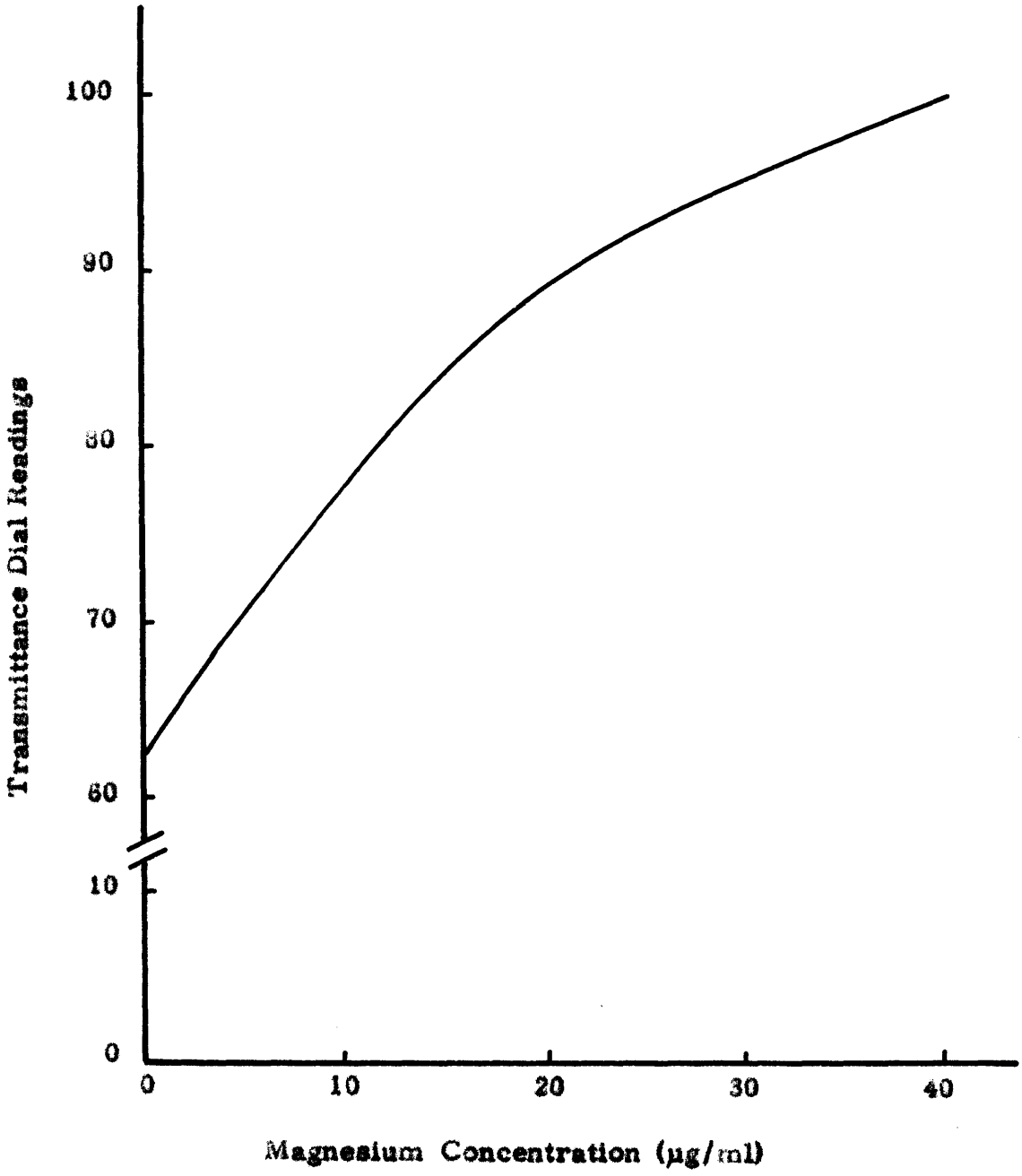

Figure 1. Standard curve for magnesium solutions containing .02 mg calcium per ml.

concentrations below 10 µg per ml. Reproducibility was not altered. Addition of these interfering cations to the standard solution to compensate for equivalent amounts in some of the samples appeared to have no adverse effect on the relationship between radiation emission and magnesium concentration in the standard solutions.

The standard curves indicated that greatest sensitivity of response to changes in magnesium ion concentration might be expected below 10 µg per ml. However, preliminary analyses of very dilute solutions showed such variability that dilution of samples to contain approximately 10 µg magnesium per ml seemed advisable. Inclusion in each series of determinations of other samples having double the low concentrations was necessary if adequacy of compensation for cation interference was to be studied. Solutions of ash obtained from food, feces, and urine composites were chosen for analysis in order to determine the accuracy and reproducibility of magnesium analysis for all three types of biological materials. Any method of analysis used in metabolic balance studies must be applicable to feces and urine samples as well as food.

Results of replicate analyses for magnesium using standard solutions containing .02 mg calcium per ml are presented in Table I. Solutions of ash designated as Food A and B, Feces E and F, and Urine J and K were those prepared from period composites collected

TABLE I

REPRODUCIBILITY OF MAGNESIUM DETERMINATIONS IN ASH SOLUTIONS OF FOOD, FECES, AND URINE WHEN STANDARD SOLUTIONS CONTAIN ADDED CALCIUM

Solution of ash	Dilution	Magnesium (µg/ml)			
		1	2	3	4
		Food			
A	1:2	25.0	20.8	28.5	24.0
	1:4	11.5	8.5	11.8	11.6
B	1:2	39.5	34.5	38.0	40.0
	1:4	17.3	17.0	20.0	18.0
		Feces			
E	1:5	16.7	12.8	16.9	16.9
	1:10	7.2	6.3	8.0	8.5
F	1:5	16.5	16.4	15.0	18.0
	1:10	6.2	5.9	7.2	5.5
		Urine			
J	1:2	19.2	17.0	19.0	18.5
	1:4	9.9	8.9	10.4	7.2
K	1:2	19.3	17.5	20.0	18.3
	1:4	10.2	9.0	11.6	6.5

in a metabolic balance experiment. The concentrations of these solutions were suitable for calcium analysis. For flame photometric magnesium determinations, the solutions were further diluted as indicated and each solution was analyzed at two dilutions. Analyses of food ash solutions were not reproducible at either concentration. Maximum difference in the apparent magnesium concentration in the series of replications for Food A (1:2) was 38 per cent of the low value. Determinations on the same sample at the 1:4 dilution differed by 36 per cent. In the case of Food B, agreement between the replicate analyses of the more dilute solutions appeared no better than that found for solutions with twice as much magnesium. Moreover, the apparent concentration of the solution of ash was dependent on the amount of magnesium present in the sample analyzed because in every case estimates made on the more dilute solutions were less than half that for the more concentrated. Differences of one to 19 per cent were obtained. In the case of solutions from feces and urine, agreement between replicate analyses was no better and apparent magnesium concentration determined was again dependent upon the magnesium level in the solutions analyzed.

In Table II are presented data from replicate analyses of more dilute solutions of ash together with those obtained when aliquots contained 5 or 10 µg of added magnesium per ml. From

TABLE II

RECOVERIES OF ADDED STANDARD IN ASH SOLUTIONS OF FOOD, FECES, AND URINE WHEN STANDARD SOLUTIONS CONTAIN ADDED CALCIUM

Solution of ash	Magnesium (μg/ml) Sample analysis 1	2	3	4	Standard added	Amount found 1	2	3	4	Average recovery %
					Food					
A	11.5	8.5	11.8	11.6	5	14.8	12.8	16.5	16.9	99±31
					10	18.5	18.3	22.5	22.4	96±18
B	17.8	17.0	20.0	18.0	5	21.1	25.8	21.2	26.7	108±75
					10	24.8	35.8	30.0	31.0	121±48
					Feces					
E	7.2	6.8	8.0	6.5	5	12.2	10.0	11.2	13.5	92±36
					10	14.2	14.8	18.0	19.0	96±25
F	6.2	5.9	7.2	5.5	5	11.5	9.7	11.2	13.3	104±38
					10	13.8	14.3	18.5	19.5	103±29
					Urine					
J	9.9	8.9	10.4	7.2	5	13.4	11.9	15.5	14.5	94±38
					10	17.2	17.0	20.8	20.0	96±24
K	10.2	9.0	11.8	8.5	5	13.0	11.9	16.0	14.0	88±43
					10	16.2	18.3	19.5	19.0	89±27

the difference between the apparent magnesium concentration of the recovery sample and that obtained from analysis of the dilute solutions in the same series, percentage recovery for each replication was calculated. Average recoveries ranged from 88 per cent in the case of Urine K to 121 per cent in the case of Food B. Far more serious, however, was the fact that differences between the replicate determinations were tremendous. As might have been expected, the standard deviation of the mean recovery was in all cases smaller when the larger amount of standard was added, but even then standard deviations were between 18 and 49 per cent when average recoveries were 96 and 121 per cent. Again, analyses for one type of sample appeared little better than those for the other two.

The apparent magnesium concentrations of the original solutions of ash calculated from analyses of each solution at two dilutions are compared in Table III with those previously determined by the thiazole yellow colorimetric method (Hunt, 1963). When the more concentrated solutions used in the present study were considered, analyses of solutions of ash from food obtained by the two methods were in excellent agreement. Results of the colorimetric analyses were only slightly higher than those of the present study. Differences were considerably greater if results obtained in analysis of the more dilute solutions were compared with the colorimetric

TABLE III

COMPARISON OF MAGNESIUM DETERMINATIONS BY TWO METHODS AND RECOVERY OF ADDED STANDARD IN ASH SOLUTIONS WHEN STANDARD SOLUTIONS CONTAIN ADDED CALCIUM

Solution of ash	Dilution	Colorimetric analysis	Flame photometric analysis	Difference	Recovery added standard[b] 5 µg	10 µg
		µg Mg/ml		%	%	
Food A	1:2	50.2	49.0±6.5[a]	-2.4		
	1:4		43.2±6.1		91±7	92±8
Food B	1:2	76.9	76.0±5.0	-1.2		
	1:4		72.8±5.1		97±13	105±20
Feces E	1:5	86.7	79.1±10.1	-9.6		
	1:10		71.2±3.0		90±7	94±12
Feces F	1:5	64.8	83.4±6.1	+22.3		
	1:10		62.0±7.3		87±8	91±15
Urine J	1:2	38.5	36.8±2.0	-4.6		
	1:4		36.3±5.9		95±10	97±9
Urine K	1:2	35.6	38.0±2.3	+6.3		
	1:4		37.3±6.7		93±9	94±8

[a]Standard deviation.

[b]Sample concentration used in calculation was one-half analyzed value for 1:2 dilution of solution of ash.

analyses. The agreement between the colorimetric estimate and the average apparent magnesium concentration of urine determined at two levels in the solutions of ash was satisfactory. Differences of about 6 per cent between determinations were found. In the case of fecal samples, agreement between the results obtained in the two methods was poor. For sample E, results of the colorimetric analysis were 10 per cent higher and for sample F, 22 per cent lower than those of the flame photometric determinations.

In the calculation of recovery of magnesium added to solutions of ash presented in Table II, the difference between the apparent magnesium concentration of the dilute solution of ash and that containing added standard was considered the amount recovered. Since almost invariably the amount found in the more dilute solution was less than half that of the more concentrated, it seemed probable that the influence of magnesium concentration on its determination was a reason for unsatisfactory recoveries. Consequently, recoveries were recalculated using the difference between the analyses of the recovery sample and one-half that of the more concentrated solution of ash. Results are included in Table III. Average recoveries calculated in this manner would indicate greater accuracy than the previous results. For ash solutions of food, average recoveries were from 91 to 105 per cent and standard deviations

ranged from 7 to 20 per cent. Feces samples had lower percentage recoveries and standard deviations were no better than for food samples. Average recoveries for Urine J were 95±10.5 and 97±8.3 per cent but for sample K recovery dropped to 93±9.2 and 94±8.1 per cent.

Except for Feces F, agreement between analyses by the colorimetric method and average apparent magnesium values obtained by the flame photometric method were acceptable. However, lack of reproducibility of analyses and dependence of the apparent magnesium concentration on the particular dilution of the sample analyzed indicated that the method was unsuitable for analysis of samples of food, feces, and urine, possibly because of interference by ions other than calcium in the solution of ash. Two ions other than calcium present in large amounts in the biological samples analyzed were sodium and potassium. Both emit strong radiation and interfere with magnesium determination through increases in background. The sodium line at 285.3 mμ is so close to the magnesium line that interference by sodium seemed a possible cause for difficulty.

A new group of standard solutions was prepared in which sodium and calcium were present in constant amounts. The quantity of sodium used by Alcock et al. (1960) in a compensating solution and that estimated to be in the solutions of ash was 0.3 mg per ml.

However, because of difficulties encountered with clogging of the burner in the preliminary study of solutions in which sulfate and phosphate had not been removed, it seemed inadvisable to add any salts at concentrations higher than necessary. Therefore, sodium was added to the standards at only .03 mg per ml. Values for replicate analyses for magnesium using the standard solutions containing added calcium and sodium are listed in Table IV. Solutions of ash of food analyzed at two dilutions varied in apparent concentration by as much as 30 per cent. In solutions from food, the apparent concentration of magnesium determined on the more dilute sample again failed to agree with that obtained with the more concentrated. However, estimates were sometimes greater and sometimes less. Samples of ash solutions of feces and urine gave no better agreement between replications and differences in estimated magnesium concentrations obtained at two dilutions were from 0 to 29 per cent.

Replicate values for samples containing 5 or 10 µg of added magnesium per ml of solution and of the dilute samples without added standard are presented in Table V. Recoveries were calculated from the difference between apparent magnesium concentration of the recovery samples and that obtained from analysis of the dilute solution in the same series. Average recoveries ranged from 88±22 per cent for Food B to 152±49 per cent for Feces F. The average recovery

TABLE IV

REPRODUCIBILITY OF MAGNESIUM DETERMINATIONS IN ASH SOLUTIONS OF FOOD, FECES, AND URINE WHEN STANDARD SOLUTIONS CONTAIN ADDED SODIUM[a] AND CALCIUM

Solution of ash	Dilution	Magnesium (µg/ml) 1	2	3	4
		Food			
A	1:2	26.0	24.0	23.0	23.0
	1:4	11.0	11.0	14.0	10.5
B	1:2	35.5	40.0	29.5	38.5
	1:4	20.0	21.5	21.0	18.5
		Feces			
E	1:5	18.0	17.0	17.5	14.0
	1:10	7.5	8.5	10.5	7.5
F	1:5	15.5	14.0	18.5	12.5
	1:10	5.5	8.0	10.0	7.0
		Urine			
J	1:2	17.5	18.5	20.5	17.0
	1:4	8.0	9.0	12.0	8.0
K	1:2	16.5	19.0	21.0	19.0
	1:4	8.5	9.0	12.0	9.0

[a]Standard solution contains .03 mg sodium per ml.

TABLE V

RECOVERIES OF ADDED STANDARD IN ASH SOLUTIONS OF FOOD, FECES, AND URINE WHEN STANDARD SOLUTIONS CONTAIN ADDED SODIUM[a] AND CALCIUM

Solution of ash	Magnesium (µg/ml) Sample analysis 1	2	3	4	Standard added	Amount found 1	2	3	4	Average recovery %
					Food					
A	11.0	11.0	14.0	10.5	5	16.5	15.0	19.0	14.0	118±14
					10	26.0	21.5	24.0	23.0	122±17
B	20.0	21.5	21.0	18.5	5	27.5	28.5	25.0	26.0	38±22
					10	32.5	40.0	32.0	36.5	95±10
					Feces					
E	7.5	8.5	10.5	7.5	5	13.5	11.0	15.5	12.5	148±42
					10	21.5	18.5	22.0	17.0	141±22
F	8.5	8.0	10.0	8.0	5	13.5	10.5	15.5	10.5	152±49
					10	20.0	14.0	20.0	16.0	135±35
					Urine					
J	8.0	9.0	12.0	8.0	5	14.5	12.5	16.0	13.0	148±32
					10	21.0	17.5	20.5	18.5	128±20
K	8.5	9.0	12.0	9.0	5	13.5	13.0	16.5	13.5	130±14
					10	20.0	18.0	21.0	20.5	120±12

[a]Standard solution contains .03 mg sodium per ml.

was above 115 per cent for all samples except Food B. Replications of recovery were no better than those shown in Table II and analyses for one type of sample appeared little better than those for the other two. A comparison was made of analyses of solutions of ash as determined by the thiazole yellow colorimetric method (Hunt, 1963) with the flame photometric method in Table VI. The former were somewhat higher than results from the flame photometric method. Average magnesium concentration of food samples found by the two methods differed by less than 5 per cent. In the case of fecal samples and Urine J, results varied by 5-10 per cent while in the case of Urine K agreement was excellent.

Recoveries were recalculated as before by using the difference between the analysis of the recovery sample and one-half that of the more concentrated solution of ash. Average recovery was 94±12 to 126±7 per cent of the magnesium added. Recoveries of 5 µg added magnesium were slightly better than of 10 µg per ml.

Because results appeared no better than previously, it was decided to use a larger amount of sodium in the standards. Therefore magnesium standards containing added calcium and 0.3 mg sodium per ml were prepared. Reproducibility of analyses for solutions of ash at two dilutions may be seen in Table VII. Replicate analyses were in no better agreement than in previous determinations

TABLE VI

COMPARISON OF MAGNESIUM DETERMINATIONS BY TWO METHODS AND RECOVERY OF ADDED STANDARD IN ASH SOLUTIONS WHEN STANDARD SOLUTIONS CONTAIN ADDED SODIUM[a] AND CALCIUM

Solution of ash	Dilution	Colorimetric analysis	Flame photometric analysis	Difference	Recovery added standard[c] 5 µg	10 µg
		µg Mg/ml		%	%	
Food A	1:2	50.2	48.4±2.6[b]	-3.7		
	1:4		45.6±5.9		94±12	118±25
Food B	1:2	73.9	73.4±8.8	-4.8		
	1:4		83.8±7.4		119±12	126±7
Feces E	1:5	86.7	82.5±7.9	-5.1		
	1:10		82.0±13.6		97±11	110±16
Feces F	1:5	64.8	71.5±8.4	+9.4		
	1:10		75.0±16.4		100±11	110±23
Urine J	1:2	33.5	35.0±4.7	-10.0		
	1:4		36.0±6.9		109±24	118±38
Urine K	1:2	35.6	36.0±5.0	+1.1		
	1:4		37.6±5.9		110±26	123±46

[a] Standard solution contains .03 mg sodium per ml.

[b] Standard deviation.

[c] Sample concentration used in calculation was one-half analyzed value for 1:2 dilution of solution of ash.

TABLE VII

REPRODUCIBILITY OF MAGNESIUM DETERMINATIONS IN ASH SOLUTIONS OF FOOD, FECES, AND URINE WHEN STANDARD SOLUTIONS CONTAIN ADDED SODIUM[a] AND CALCIUM

Solution of ash	Dilution	Magnesium (µg/ml)		
		1	2	3
		Food		
A	1:2	20.0	23.0	23.5
	1:4	9.0	10.0	10.0
B	1:2	35.0	32.5	37.0
	1:4	15.0	16.5	19.0
		Feces		
E	1:5	11.5	15.0	14.5
	1:10	5.5	7.5	7.5
F	1:5	10.5	14.5	13.0
	1:10	6.0	6.5	7.0
		Urine		
J	1:2	12.0	15.0	15.5
	1:4	7.5	7.5	8.0
K	1:2	13.5	16.0	16.0
	1:4	8.0	7.5	8.5

[a]Standard solution contains .3 mg sodium per ml.

and the type of material had no influence on analysis. In the case of solutions of ash from food the maximum value exceeded the lowest by 11 to 27 per cent and for solutions from feces and urine by 17 to 38 and 7 to 29 per cent, respectively. Differences between replicate analyses of the more dilute solutions were in general as great as those found for the more concentrated. The apparent magnesium concentration of solutions of ash obtained by analysis at two dilutions differed by 0 to 25 per cent. While 10 of the 18 comparisons agreed within 10 per cent, there was no consistent pattern. Differences of 0, 3, and 25 per cent were found in the replicate analysis of Urine J. Results of replicate analyses of samples containing 5 or 10 μg of added magnesium per ml together with analyses of samples to which no standard had been added are listed in Table VIII. Percentage recovery of the added standard is presented also. Reproducibility did not improve with any group of samples. Average recoveries ranged from 77±40 per cent for Urine K to 118±43 per cent for Food B. Standard deviations were larger on the average than in previous determinations. Calculations of recovery of 5 and 10 μg magnesium added per ml were made using the difference between the analysis of the recovery sample and one-half that of the more concentrated solution of ash (Table IX). Average recoveries ranged from 93±10 per cent to 99±15 per cent with one exception. Improvement in

TABLE VIII

RECOVERIES OF ADDED STANDARD IN ASH SOLUTIONS OF FOOD, FECES, AND URINE WHEN STANDARD SOLUTIONS CONTAIN ADDED SODIUM[a] AND CALCIUM

Solution of ash	Magnesium (ug/ml) Sample analysis 1	2	3	Standard added	Amount found 1	2	3	Average recovery %
				Food				
A	9.0	10.0	10.0	5	12.0	15.5	14.5	93±35
				10	16.0	23.0	22.0	107±32
B	15.0	16.5	19.0	5	16.0	26.0	22.0	90±88
				10	21.5	32.5	32.0	116±48
				Feces				
E	5.5	7.5	7.5	5	9.5	13.0	12.0	93±15
				10	12.5	19.0	16.5	92±22
F	6.0	6.5	7.0	5	8.0	13.5	10.5	83±51
				10	12.0	16.5	16.5	85±21
				Urine				
J	7.5	7.	8.0	5	9.5	14.5	12.0	87±50
				10	12.5	19.0	18.0	88±34
K	8.0	7.5	8.5	5	10.0	13.5	12.0	77±40
				10	14.0	20.0	18.5	95±32

[a]Standard solution contains .3 mg sodium per ml.

TABLE IX

COMPARISON OF MAGNESIUM DETERMINATIONS BY TWO METHODS AND RECOVERY OF ADDED STANDARD IN ASH SOLUTIONS WHEN STANDARD SOLUTIONS CONTAIN ADDED SODIUM[a] AND CALCIUM

Solution of ash	Dilution	Colorimetric analysis	Flame photometric analysis	Difference	Recovery added standard[c] 5 µg	10 µg
		µg Mg/ml		%	%	
Food A	1:2	50.2	44.3±3.8[b]	-13.3		
	1:4		38.7±2.3		89±10	96±14
Food B	1:2	76.9	69.7±4.5	-10.3		
	1:4		67.3±8.1		96±26	125±47
Feces E	1:5	86.7	68.3±9.5	-26.9		
	1:10		68.3±11.3		97±8	94±14
Feces F	1:5	64.8	53.3±10.1	-2.4		
	1:10		65.0±5.0		94±16	92±11
Urine J	1:2	38.5	28.3±3.8	-36.0		
	1:4		30.7±1.2		99±15	96±16
Urine K	1:2	35.6	30.3±2.9	-17.5		
	1:4		32.0±2.0		94±10	99±14

[a]Standard solution contains .3 mg sodium per ml.

[b]Standard deviation.

[c]Sample concentration used in calculation was one-half analyzed value for 1:2 dilution of solution of ash.

recovery calculated by this method again indicated that apparent magnesium concentration is dependent on the concentration of the sample analyzed.

A comparison of average magnesium concentrations of solutions of ash determined by the thiazole yellow colorimetric method (Hunt, 1963) and the flame photometric method may be found in Table IX. The flame photometric determinations were lower than the colorimetric values by 2.4 to 36.0 per cent. Except for Feces F, differences in magnesium concentration determined by the two methods were considerably greater than those obtained when calcium was the only interfering ion present in the standards. Evidently addition of sodium ions to the standards to compensate for that in the samples failed to reduce interference in the magnesium determination. Since potassium interferes also the possibility that inclusion of potassium chloride together with sodium and calcium chlorides in the standards might compensate for interfering ions in the solutions of ash was investigated.

The amount of potassium added was that used by Alcock et al. (1960). With this inclusion, the cations added to standard solutions of magnesium were present in the same amounts as those found effective by the British investigators in overcoming interference and the anions (phosphate and sulfate) had been removed by precipitation. Replicate

analyses of solutions of ash at two dilutions are presented in Table X. Maximum differences between replicate analyses were 25 to 50 per cent of the lowest value. Lack of reproducibility was not influenced by whether the solution of ash was prepared from food, feces, or urine. Influence of the level of magnesium in the solution analyzed was still evident. Differences in the apparent concentration obtained at two dilutions varied from 0 to 40 per cent and were unrelated to the nature of the biological material represented. For example, results of analysis of solutions of Feces F varied from complete agreement to differences of 4 and 22 per cent and in Food B from 1 to 25 per cent. Replicate analyses of the dilute solutions of ash and those containing an added 10 μg magnesium per ml are listed in Table XI. Average recoveries, calculated from the differences between the apparent magnesium concentration of the recovery sample and that obtained from analysis of the dilute solution in the same series, were between 93±21 and 107±20 per cent. Although the average percentage recovery of added magnesium would suggest reasonably good accuracy, the large standard deviations indicate the unreliability of individual analyses. Results do suggest, however, that accuracy was slightly increased by the inclusion of potassium as well as sodium chlorides in the standard solutions. Calculations of recovery based on analysis of the less dilute solutions are given in Table XII. Apparent recovery

TABLE X

REPRODUCIBILITY OF MAGNESIUM DETERMINATIONS IN ASH SOLUTIONS OF FOOD, FECES, AND URINE WHEN STANDARD SOLUTIONS CONTAIN ADDED POTASSIUM, SODIUM, AND CALCIUM

Solution of ash	Dilution	Magnesium (µg/ml)		
		1	2	3
		Food		
A	1:2	25.5	17.0	20.0
	1:4	10.0	7.5	7.0
B	1:2	----	27.5	40.0
	1:4	19.0	13.5	15.0
		Feces		
E	1:5	15.5	12.0	13.5
	1:10	7.0	4.5	5.0
F	1:5	13.5	10.0	11.5
	1:10	7.0	5.0	4.5
		Urine		
J	1:2	19.0	14.5	18.5
	1:4	10.0	7.5	8.5
K	1:2	20.0	16.0	20.0
	1:4	8.0	8.5	6.0

TABLE XI

RECOVERIES OF ADDED STANDARD IN ASH SOLUTIONS OF FOOD, FECES, AND URINE WHEN STANDARD SOLUTIONS CONTAIN ADDED POTASSIUM, SODIUM, AND CALCIUM

Solution of ash	Magnesium (µg/ml) Sample analysis 1	2	3	Standard added	Amount found 1	2	3	Average recovery %
				Food				
A	10.0	7.5	7.0	10	21.0	14.5	17.5	95±21
B	19.0	13.5	15.0	10	33.0	20.0	24.0	98±38
				Feces				
E	7.0	4.5	5.0	10	18.0	12.0	16.0	98±20
F	7.0	5.0	4.5	10	17.0	14.5	15.0	100±5
				Urine				
J	10.0	7.5	6.5	10	20.0	14.5	17.5	93±21
K	8.0	8.5	6.0	10	19.0	17.0	18.5	107±20

TABLE XII

COMPARISON OF MAGNESIUM DETERMINATIONS BY TWO METHODS AND RECOVERY OF ADDED STANDARD IN ASH SOLUTIONS WHEN STANDARD SOLUTIONS CONTAIN ADDED POTASSIUM, SODIUM, AND CALCIUM

Solution of ash	Dilution	Colorimetric analysis	Flame photometric analysis	Difference	Recovery added standard[b] 10 µg
		µg Mg/ml		%	%
Food A	1:2	50.2	41.7±8.6[a]	-20.4	86±7
	1:4		32.7±6.4		
Food B	1:2	76.9	87.5±17.4	-13.9	
	1:4		83.3±12.1		82±3
Feces E	1:5	86.7	68.3±8.8	-26.9	
	1:10		55.0±13.1		90±13
Feces F	1:5	64.8	58.0±8.3	-11.7	
	1:10		55.0±13.1		98±3
Urine J	1:2	38.5	34.7±4.9	-11.0	
	1:4		32.0±7.2		93±9
Urine K	1:2	35.8	37.3±4.6	+4.6	94±1
	1:4		30.0±5.3		

[a]Standard deviation.

[b]Sample concentration used in calculation was one-half analyzed value for 1:2 dilution of solution of ash.

was now 82±3 to 98±3 per cent. Recoveries calculated in this manner were better for solutions of ash from feces and urine than from food and agreement between replications was strikingly improved. Except for Urine K, magnesium determinations by the flame photometric method were from 11 to 27 per cent below those obtained by colorimetric analysis (Table XII). Disagreement would have been even larger had the comparisons been based on the more dilute solutions of ash. It is evident, therefore, that in this series of analyses also the apparent magnesium concentration determined was dependent upon its level in the solution analyzed. This fact together with lack of agreement between individual determinations indicates that interference by ions other than magnesium in the solutions of ash was not eliminated.

Stannic chloride used to remove phosphate and sulfate ions was added in equal amount per ml to solutions of ash and standards; the excess stannic ion theoretically was completely removed by precipitation of gelatinous stannic acid. Nevertheless, there is a possibility that removal was incomplete and that differing amounts of stannic ion remaining in the solutions might have caused interference. For this reason, a final study was conducted in which ferric and barium ions were substituted for stannic ions for the precipitation of phosphate and sulfate. Calcium, sodium, and potassium chlorides

were present in the standard solutions in the same concentrations as in the previous series. These standard curves were similar to those obtained when stannic chloride was the precipitating agent. As before, sensitivity was greatest in the range of 0 to 10 µg magnesium per ml and lowest above 20 µg magnesium per ml. Replicate readings showed greater variation than was found previously.

Data obtained in replicate analyses of solutions of ash from food, feces, and urine for magnesium are presented in Table XIII. Solutions of ash designated as Food C and D, Feces G and H, and Urine L and M were period composites collected in a metabolic balance experiment and were similar to those used in earlier parts of this work. Dilutions are indicated in Table XIII. Differences in analyses of a particular sample ranged from 11 to 56 per cent of the lower value in solutions from food and feces and from 20 to 120 per cent in ash solutions from urine. Complete lack of reproducibility in the urine analyses suggests possible failure to completely remove phosphate present in greater amount in the urine composites than in food and feces. However, ferric chloride was added 70 per cent in excess of the amount required. The amount of barium chloride added was 70 per cent in excess of that needed to precipitate the sulfate estimated to be present. Magnesium concentration of solutions of ash obtained from the more dilute solutions was in

TABLE XIII

REPRODUCIBILITY OF MAGNESIUM DETERMINATIONS IN ASH SOLUTIONS OF FOOD, FECES, AND URINE[a] WHEN STANDARD SOLUTIONS CONTAIN ADDED POTASSIUM, SODIUM, AND CALCIUM

Solution of ash	Dilution	Magnesium (ug/ml)			
		1	2	3	4
		Food			
C	1:2	17.0	19.0	17.5	19.0
	1:4	12.5	14.0	11.5	11.5
D	1:2	18.0	18.0	20.0	21.5
	1:4	13.5	12.0	14.0	13.0
		Feces			
G	1:5	11.5	8.0	8.0	12.5
	1:10	5.5	5.5	5.5	7.0
H	1:5	9.0	10.0	10.0	10.0
	1:10	6.0	5.0	6.0	5.5
		Urine			
L	1:2	5.0	3.5	4.0	4.0
	1:4	3.0	2.5	2.5	3.0
M	1:2	5.5	2.5	2.5	3.5
	1:4	2.5	1.5	2.0	2.0

[a]Phosphate and sulfate removed by ferric and barium chlorides.

general higher than from the more concentrated. Maximum differences found were from 0 to 60 per cent. Replicate values for samples containing added magnesium and those without added magnesium in the same series are shown in Table XIV. Average recoveries represented 83±12 to 138±27 per cent of the added magnesium. One type of sample gave no better results than the other two. Recoveries calculated from one-half the magnesium found in the more concentrated samples are presented in Table XV. For urine the added standard recovered was low, 68 and 85 per cent. With the exception of Feces H, recovery of magnesium added to solutions of food and feces was high and ranged from 101±9 to 119±10.

Magnesium concentrations of all solutions of ash were lower than those obtained in any of the series in which stannic chloride was the precipitating agent. Values obtained in the last analyses (Table XV) were 76 to 534 per cent lower than the amounts found by colorimetric analysis. In every respect, substitution of ferric and barium chlorides for stannic chloride in the removal of interfering anions resulted in less satisfactory magnesium determinations. For this reason, further investigation of the procedure was abandoned.

On the basis of the criteria established, it appeared that analyses made with standards to which only calcium had been added gave the best results. However, determinations of magnesium

TABLE XIV

RECOVERIES OF ADDED STANDARD IN ASH SOLUTIONS OF FOOD, FECES, AND URINE[a] WHEN STANDARD SOLUTIONS CONTAIN ADDED POTASSIUM, SODIUM, AND CALCIUM

Solution of ash	Magnesium (μg/ml)									Average recovery %
	Sample analysis				Standard added	Amount found				
	1	2	3	4		1	2	3	4	
					Food					
C	12.5	14.0	11.5	11.5	10	19.0	18.0	24.0	21.5	110±37
D	13.5	12.0	14.0	13.0	10	20.0	22.5	25.5	26.0	138±27
					Feces					
G	5.5	5.5	5.5	7.0	10	14.0	14.5	15.0	17.0	123±6
H	6.0	5.0	6.0	5.5	10	12.0	13.5	16.0	14.0	110±16
					Urine					
L	3.0	2.5	2.5	3.0	10	9.5	9.5	11.5	10.5	100±11
M	2.5	1.5	2.0	2.0	10	9.5	7.0	6.5	9.0	83±12

[a]Phosphate and sulfate removed by ferric and barium chlorides.

TABLE XV

COMPARISON OF MAGNESIUM DETERMINATIONS BY TWO METHODS AND RECOVERY OF ADDED STANDARD IN ASH SOLUTIONS[a] WHEN STANDARD SOLUTIONS CONTAIN ADDED POTASSIUM, SODIUM, AND CALCIUM

Solution of ash	Dilution	Colorimetric analysis	Flame photometric analysis	Difference	Recovery added standard[c] 10 μg
		μg Mg/ml		%	%
Food C	1:2	71.7	36.2±2.1[b]	-98.1	
	1:4		49.5±4.7		108±14
Food D	1:2	58.2	38.8±3.4	-75.3	
	1:4		52.5±3.4		119±10
Feces G	1:5	91.7	50.0±11.4	-83.4	
	1:10		58.3±7.5		101±9
Feces H	1:5	88.7	48.8±7.5	-81.8	
	1:10		56.2±4.8		93±10
Urine L	1:2	52.0	8.2±1.2	-534.1	
	1:4		11.0±1.1		85±9
Urine M	1:2	27.2	7.0±2.8	-288.6	
	1:4		8.0±1.6		66±9

[a]Phosphate and sulfate removed by ferric and barium chlorides.

[b]Standard deviation.

[c]Sample concentration used in calculation was one-half analyzed value for 1:2 dilution of solution of ash.

concentration of the solutions of ash were dependent on the level in the sample analyzed, and lack of reproducibility of replicate analyses indicated that the method was unsuitable for use in the routine analysis of biological materials characterized by considerable differences in concentration. Even if conditions had been found permitting satisfactory analysis, use of such a flame photometric procedure had doubtful advantage over the colorimetric procedure with regard to time expended. The time consuming step in the latter is the preparation of samples for color development. Procedures necessary for the removal of phosphate and sulfate ions in the flame photometric method required fully as much expenditure of time.

CHAPTER V

SUMMARY

The study reported in this paper was undertaken to investigate flame photometric determinations of magnesium in solutions of ash from biological samples collected during metabolic balance experiments. Two general methods for elimination of interference by other ions were studied. One was that of Alcock et al. (1960) in which adverse effects were eliminated by addition of anions and cations to the standard solutions in amounts comparable to the levels in samples to be analyzed. However, this method was abandoned because the atomizer burner of the flame photometer became too badly clogged for further use. Poorly volatilized calcium sulfate and phosphate may have been responsible.

The other method depends on removal of interfering anions by precipitation and addition of interfering cations to the standard solution. The first procedure investigated involved removal of phosphate and sulfate ions by precipitation with stannic chloride and addition of salts of calcium, of calcium and sodium, and finally of calcium, sodium, and potassium to the standards to compensate for the varying amounts present in the samples. Reproducibility, recovery of added

magnesium, and agreement of values obtained from flame photometric and colorimetric determinations were criteria for evaluation of the method. Standard curves were reproducible in all series of determinations, but replicate analyses of solutions were in poor agreement. Apparent magnesium concentration of solutions of ash and recovery of added standard were dependent on the amount of magnesium in the sample actually analyzed. Agreement of magnesium concentrations obtained by the two methods was satisfactory only for the series in which calcium alone was added to the standard solutions.

Precipitation of phosphate and sulfate ions with barium and ferric chlorides from standards and samples was the other procedure studied. In one series of determinations, calcium, sodium, and potassium chlorides were added to the standards to compensate for the amounts found in the samples. Reproducibility and recovery of added magnesium were no better than those obtained by the previous determinations. Influence of the concentration of the solution analyzed upon the apparent magnesium concentration of the original solution of ash was not eliminated. Results obtained by the flame photometric method were in every case much less than those from colorimetric determinations.

The procedures for flame photometric determination of magnesium in ash from food, feces, and urine samples were generally unsatisfactory judged by all of the criteria studied. Processes required for removal of the interfering anions and addition to the standards of interfering cations were so time consuming that the flame photometric method studied had little advantage over the colorimetric method in this respect.

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